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11. [6355420](#). 13 Aug 98; 12 Mar 02. Methods and products for analyzing polymers. Chan; Eugene Y.. 435/6; 435/149 435/287.1 435/287.2 435/94 435/970 435/973. C12Q001/68.

---

12. [6294136](#). 13 May 97; 25 Sep 01. Image processing and analysis of individual nucleic acid molecules. Schwartz; David C.. 422/186; 422/129 422/55 422/58 422/99 435/6 536/23.1. B01J019/08 C12Q001/68 C07H021/02.

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13. [6271042](#). 26 Aug 98; 07 Aug 01. Biochip detection system. Watson, Jr.; Robert Malcolm, et al. 436/172; 250/458.1 250/459.1 422/82.08. G01N021/64.

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14. [6270730](#). 16 Jun 98; 07 Aug 01. Multi-well rotary synthesizer. McLuen; Gary R., et al. 422/131; 422/62 422/81. C08F283/00.

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15. [6221592](#). 20 Oct 98; 24 Apr 01. Computer-based methods and systems for sequencing of individual nucleic acid molecules. Schwartz; David C., et al. 435/6; 435/91.1. C12Q001/68 C12P019/34.

---

16. [6210896](#). 13 Aug 99; 03 Apr 01. Molecular motors. Chan; Eugene Y.. 435/6; 422/50 422/82.01 436/156 436/94. C12Q001/68 G01N033/00 G01N025/54.

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17. [6150173](#). 15 Jul 99; 21 Nov 00. Automated determining and measuring device and method. Schubert; Walter. 436/43; 422/100 422/63 422/65 422/67 422/68.1 422/82.08 436/164 436/172 436/174 436/180 436/47 436/48 436/49 436/50. G01N035/00.

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18. [6147198](#). 03 Apr 95; 14 Nov 00. Methods and compositions for the manipulation and characterization of individual nucleic acid molecules. Schwartz; David C.. 536/23.1; 536/24.3. C07H021/04 C07H021/02.

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19. [5720928](#). 03 Apr 95; 24 Feb 98. Image processing and analysis of individual nucleic acid molecules. Schwartz; David C.. 422/186; 422/129 422/55 422/58 422/99 435/6. B01J019/08 G01N021/00 G01N031/22 B01L003/00.

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L1: Entry 15 of 19

File: USPT

Apr 24, 2001

US-PAT-NO: 6221592

DOCUMENT-IDENTIFIER: US 6221592 B1

TITLE: Computer-based methods and systems for sequencing of individual nucleic acid molecules

DATE-ISSUED: April 24, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schwartz; David C.	New York	NY		
Mishra; Bhubaneswar	Great Neck	NY		

US-CL-CURRENT: 435/6; 435/91.1

## CLAIMS:

What is claimed is:

1. A method for imaging a single labeled nucleotide on an individual double stranded nucleic acid molecule, comprising:
  - (a) nicking a double stranded nucleic acid molecule elongated and fixed onto a surface so that the double stranded nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of a labeled nucleotide creating a nicked strand;
  - (b) enzymatically adding a single nucleotide comprising a label; and
  - (c) imaging the added label.
2. The method of claim 1, in which the step of nicking the double stranded nucleic acid molecule is performed by the enzyme DNase.
3. The method of claim 1, in which the surface is a planar surface.
4. The method of claim 1, in which the step of adding nucleotides comprising a label is performed by a polymerase.
5. The method of claim 4, in which the polymerase is DNA Polymerase I, the Klenow fragment of DNA Polymerase I lacking the 5'-3' exonuclease activity, T7 Sequenase v. 2.0 or Taq polymerase.
6. The method of claim 1, in which the step of imaging the label is performed using a camera and a microscope.
7. The method of claim 6, in which the step of imaging the label further comprises using laser illumination.
8. The method of claim 1, in which the step of imaging further comprises using a computer.
9. The method of claim 1, further comprising analyzing the image using a mathematical algorithm.

10. The method of claim 9, in which the mathematical algorithm is a Bayesian estimation method.

11. The method of claim 1, further comprising the steps:

(a) modifying the label after imaging the label in order to visualize the subsequently added labeled nucleotides; and

(b) repeating the steps of enzymatically adding a nucleotide comprising a label, imaging the label, and modifying the label to image multiple, consecutively added nucleotides.

12. The method of claim 11, further comprising enzymatically displacing the nicked strand of the nucleic acid molecule.

13. The method of claim 12, in which enzymatically displacing the nicked strand is performed using the Klenow fragment of DNA Polymerase I.

14. The method of claim 11, further comprising enzymatically opening the nicked sites on the double stranded nucleic acid molecule.

15. The method of claim 14, in which the step of opening the nicked sites is performed by an enzyme having 5'-3' exonuclease activity.

16. The method of claim 15, in which the enzyme having 5'-31 exonuclease activity is DNA Polymerase I or T7 exonuclease.

17. The method of claim 11, in which the label is photolabile.

18. The method of claim 11, in which the step of modifying the label after imaging is performed by photobleaching or photolysis.

19. A method for determining the nucleotide sequence of an individual double stranded nucleic acid molecule, comprising:

(a) nicking a double stranded nucleic acid molecule elongated and fixed onto a surface so that the double stranded nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides forming a 3' terminus to create an extended strand;

(b) extending the strand by adding at least one nucleotide comprising a label to the 3' terminus of the nicked site; and

(c) imaging the added label.

20. The method of claim 19, in which the step of nicking the double stranded nucleic acid molecule is performed by the enzyme DNase.

21. The method of claim 19, further comprising enzymatically displacing the nicked strand of the nucleic acid molecule.

22. The method of claim 21, in which enzymatically displacing the nicked strand is performed using the Klenow fragment of DNA Polymerase I.

23. The method of claim 19, further comprising opening the nicked sites on the double stranded nucleic acid molecule.

24. The method of claim 23, in which the step of opening the nicked sites on the double stranded nucleic acid molecule is performed by an enzyme having 5'-3' exonuclease activity.

25. The method of claim 24, in which the enzyme having 5'-3' exonuclease activity is DNA Polymerase I or T7 exonuclease.
26. The method of claim 19, in which the step of extending the strand by adding a nucleotide comprising a label is performed by a polymerase.
27. The method of claim 26, in which the polymerase is DNA Polymerase I, the Klenow fragment of DNA Polymerase I lacking the 5'-3' exonuclease activity, T7 Sequenase v. 2.0, or a Taq polymerase.
28. The method of claim 23, in which the step of opening the nicked sites on the double stranded nucleic acid molecule and extending the strand by adding a nucleotide comprising a label is performed by T7 exonuclease gene 6 and T7 Sequenase v. 2.0, respectively.
29. The method of claim 19, in which the step of imaging the label is performed using a camera and a microscope.
30. The method of claim 29, in which the step of imaging the label further comprises using laser illumination.
31. The method of claim 19, in which the label is photolabile.
32. The method of claim 19, further comprising modifying the label after imaging in order to visualize subsequently added labels.
33. The method of claim 3, in which the step of modifying the label after imaging is performed by photobleaching or photolysis.
34. The method of claim 19, in which the nucleotides comprise a mix of labeled and unlabeled nucleotides.
35. A system for determining the nucleotide sequence of an individual double stranded nucleic acid molecule, comprising:
  - (a) the double stranded nucleic acid molecule elongated and fixed onto a surface so that the nucleic acid molecule remains accessible for enzymatic reactions and/or hybridization reactions;
  - (b) a polymerase fixed on the surface;
  - (c) nucleotides comprising a label fixed on the surface; and
  - (d) a device for imaging the label to produce an image.
36. The system of claim 35, in which the polymerase is DNA Polymerase I, the Klenow fragment of DNA Polymerase I without the 5'-3' exonuclease activity T7 Sequenase v. 2.0, or Taq polymerase.
37. The system of claim 35, further comprising a nucleic acid nicking enzyme.
38. The system of claim 36, in which the nicking enzyme is a DNase.
39. The system of claim 35, in which the label is a fluorescent label.
40. The system of claim 35, further comprising a nick opening enzyme fixed on the surface.
41. The system of claim 40, in which the nick opening enzyme is T7 exonuclease gene 6, DNA Polymerase I, the Klenow fragment of DNA Polymerase I or a 5'-3'

exonuclease.

42. The system of claim 35, in which the device for imaging comprises a fluorescence microscope, a camera and a source of illumination.

43. The system of claim 35, in which the source of illumination is a laser.

44. The system of claim 35, in which the device for imaging the label processes the image using Bayesian estimation, comprising:

- (a) accumulating signals of an addition site of the image;
- (b) filtering the signals according to fluorescence intensity;
- (c) correlating the signals with the backbone of the nucleic acid molecule;
- (d) tabulating addition sites of the image using Bayesian inference estimation of the signals; and
- (e) aligning and assembling the addition sites to determine a nucleotide addition.

45. A method of determining the nucleotide sequence of an individual nucleic acid molecule, comprising:

(a) exposing a nucleic acid molecule annealed with at least one primer elongated and fixed onto a surface so that the nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides to a polymerase and dideoxy nucleotides comprising a base and a label; and

(b) imaging the labeled nucleotides added onto the primer;

to determine the nucleotide sequence of the nucleic acid molecule by the addition of the labeled dideoxy nucleotide.

46. A method of determining the nucleotide sequence of an individual nucleic acid molecule, comprising:

(a) exposing a nucleic acid molecule annealed with at least one primer elongated and fixed onto a surface so that the nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of a labeled nucleotide to a polymerase and nucleotides comprising a base and a label; and

(b) imaging the labeled nucleotide added onto the primers;

to determine the nucleotide sequence of the nucleic acid molecule by the addition of the labeled dideoxy nucleotide.

47. A method of determining the nucleotide sequence of an individual-nucleic acid molecule, comprising:

(a) exposing a nucleic acid molecule annealed with at least one primer elongated and fixed onto a surface so that the nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides to a polymerase and dideoxy nucleotides;

(b) exposing the nucleic acid molecule annealed with at least one primer to a polymerase and nucleotides including nucleotides comprising a label to produce a labeled primer extension nucleic acid molecule; and

(c) imaging the labeled primer extension nucleic acid molecule to produce an image;

to determine the nucleotide sequence of the nucleic acid molecule by the absence of a primer extension product corresponding to the dideoxy nucleotides used in step (a).

48. A method of determining the nucleotide sequence of an individual nucleic acid molecule, comprising:

(a) elongating and fixing the nucleic acid molecule onto a surface so that the nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides;

(b) annealing at least one primer to the elongated and fixed nucleic acid molecule;

(c) exposing the nucleic acid molecule annealed with a primer to a polymerase and dideoxy nucleotides;

(d) exposing the nucleic acid molecule to a polymerase and nucleotides including nucleotides comprising a label to produce at least one labeled primer extension nucleic acid molecule; and

(e) imaging the labeled primer extension nucleic acid molecule to produce an image;

to determine the nucleotide sequence of the nucleic acid molecule by the absence of a primer extension product corresponding to the dideoxy nucleotides used in step (c).

49. A system for determining the nucleotide sequence of an individual nucleic acid molecule, comprising:

(a) an elongated and fixed nucleic acid molecule on a surface so that the nucleic acid molecules remain accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides;

(b) at least one primer annealed to the nucleic acid molecule;

(c) a polymerase enzyme fixed on the surface to produce a primer extension product;

(d) dideoxy nucleotides fixed on the surface;

(e) nucleotides comprising a label fixed on the surface; and

(f) a device for imaging the elongated and fixed nucleic acid molecule to detect the presence of labeled nucleotides in the primer extension product to produce an image;

whereby the absence of the image of the primer extension product for a particular dideoxy nucleotide corresponds to the nucleotide sequence at one position of the nucleic acid molecule.

50. A system for determining a single nucleotide polymorphism in a population of nucleic acid molecules, comprising the system of claim 47, in which the nucleic acid molecules are elongated and fixed onto at least four surfaces and the surfaces are individually exposed to dideoxynucleotides comprising different bases.

51. A method for imaging multiple labeled nucleotides on an individual double

stranded nucleic acid molecule, comprising:

(a) nicking a nucleic acid molecule elongated and fixed onto a surface so that the double stranded nucleic acid molecule remains accessible for enzymatic reactions with enzymes for the addition of labeled nucleotides;

(b) enzymatically adding multiple nucleotides comprising at least four bases and at least four labels; and (c) simultaneously imaging the added labels.

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## Search Results - Record(s) 1 through 10 of 19 returned.

- 1. [6610256](#). 24 Sep 01; 26 Aug 03. Image processing and analysis of individual nucleic acid molecules. Schwartz; David C.. 422/186; 422/129 422/55 422/58 422/99 435/6 536/23.1. B01J019/08 C12Q001/68 C07H021/02.
- 2. [6607888](#). 19 Apr 01; 19 Aug 03. Method for analyzing nucleic acid reactions. Schwartz; David C., et al. 435/6; 435/91.1 435/91.2 536/23.1 536/24.3 536/24.33 536/25.3. C12Q001/68 C12P019/34 C07H021/02 C07H021/04 C07H021/00.
- 3. [6591196](#). 06 Jun 00; 08 Jul 03. Method and system for extracting data from surface array deposited features. Yakhini; Zohar, et al. 702/28; 356/300. G01J001/02.
- 4. [6582903](#). 10 Feb 98; 24 Jun 03. Method and a device for the evaluation of biopolymer fitness. Rigler; Rudolf, et al. 435/6; 356/302 356/306 356/311 356/319 356/320 356/335 356/36 422/55 422/58 422/99 435/287.1 435/287.2 435/288.7 435/7.1 435/808 436/34 436/43 436/517 436/518 436/805. G01N033/543.
- 5. [6509158](#). 13 Nov 00; 21 Jan 03. Image processing and analysis of individual nucleic acid molecules. Schwartz; David C.. 435/6; 436/94. C12Q001/68 G01N033/00.
- 6. [6475736](#). 25 Oct 00; 05 Nov 02. Methods for genetic analysis of DNA using biased amplification of polymorphic sites. Stanton, Jr.; Vincent P.. 435/6; 435/91.2 536/22.1 536/24.33. C12Q001/68 C12P019/34 C07H021/04 C07H019/00.
- 7. [6448012](#). 16 Nov 00; 10 Sep 02. Method for mapping a nucleic acid. Schwartz; David. 435/6; 436/94. C12Q001/68.
- 8. [6447724](#). 15 Dec 98; 10 Sep 02. DNA sequencing using multiple fluorescent labels being distinguishable by their decay times. Jensen; Morten, et al. 422/68.1; 422/55 422/58 422/82.05 422/99 435/6 435/91.1 435/91.2. G01N015/06 G01N033/00 G01N033/48 G01N021/29.
- 9. [6418238](#). 22 Sep 98; 09 Jul 02. Image detection apparatus and image detection method capable of detecting roundish shape. Shiratani; Fumiuki, et al. 382/133; 382/128. G06K009/00.
- 10. [6403311](#). 13 Aug 99; 11 Jun 02. Methods of analyzing polymers using ordered label strategies. Chan; Eugene Y.. 435/6; 435/375 435/5 436/94 546/33 548/416 549/382. C12Q001/68.

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